having a >10-fold range in E_2 (1.35 kcal/mol) were: δ (18′, 19′, 20′, 21′ and 23′) and β (21′, 24′ and 27′). These all showed approximately the same Φ value: 0.30. Comparing this value to those in the 'cap' region of other subunits, the Φ-order is $\alpha > \epsilon > \delta = \beta$. The high Φ and large energy changes are apparently only in the α subunit M2-cap, which indicates that domain plays a special role in AChR gating. NIH (NS 23513, 064969)

683-Pos

Fourier Transform Coupled Tryptophan Scanning Mutagenesis of the Lipid Exposed DM3 And DM4 Transmembrane Domains of the Torpedo Californica Acetylcholine Receptor

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The lipid-protein interface is an important domain of the acetylcholine receptor (AChR) that has recently garnered increasing relevance. Several studies have made significant advances toward determining the structure and dynamics of the lipid-exposed domains of the AChR. However, there is still a need to identify and gain insight into the mechanism through which lipid-protein interactions regulate AChR function and dynamics. In this study, we extend the Fourier Transform coupled Tryptophan Scanning Mutagenesis (FT-TrpScanM) approach to monitor the conformational changes experienced by the δM3 and δM4 transmembrane domains of the *Torpedo californica* AChR, and to identify which lipid-exposed positions on these domains are potentially linked to the regulation of ion channel kinetics. The perturbations produced by periodic tryptohan substitutions along the δM3 and δM4 transmembrane domains were characterized by two-electrode voltage clamp and ^{125}I-labeled α-bungarotoxin binding assays. The periodicity profiles and Fourier Transform spectra of these domains revealed a thinner-elongated helical structure for the closed-channel state and a thicker-shrunken helical structure for the open-channel state. The difference in oscillation patterns between the closed- and open-channel states shows a substantial conformational change along these domains as a consequence of channel activation. These results support the recently proposed spring model for the αM3 transmembrane domain of the *Mus musculus* AChR. Furthermore, the present data demonstrates that the lipid-protein interface of the AChR plays an important role in the propagation of the conformational wave needed for channel gating.

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The Nicotinic Pharmacophore - Binding Interactions in the Neuronal α4β2 Receptor

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The α4β2 nicotinic acetylcholine receptor is a pentameric, neuronal, ligand-gated ion channel that binds nicotine, acetylcholine and structurally related agonists. Pharmacophore models for nicotinic agonists have been proposed since 1970. Central to each model is the presence of a cationic nitrogen and a hydrogen bond acceptor. We have identified the binding partners for both components. Binding of the cationic nitrogen of nicotine is mediated through a cation- π interaction at αW154 in loop β of the extracellular domain as well as a hydrogen bond to the backbone carbonyl of αW154. The hydrogen bond acceptor moiety (the pyridine nitrogen of nicotine) makes a hydrogen bond to the backbone NH of L119 of the complementary subunit. These interactions were also shown to be relevant for other nicotinic agonists at both receptor stoichiometries, $(\alpha4)_2(\beta2)_3$ and $(\alpha4)_3(\beta2)_2$. Taken together, these data represent a completed nicotinic pharmacophore and offer insight into the design of new therapeutic agents that selectively target these receptors.

685-Pos

Energy Changes for Small Molecules at the Acetylcholine Receptor-Channel Transmitter Binding Site

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Acetylcholine receptors (AChRs) are ligand-gated ion channels involved in vertebrate neuromuscular transmission. Binding of acetylcholine (ACh, the endogenous neurotransmitter) increases the equilibrium constant of the 'gating' isomerization and the probability that ion channel domain adopts an ion-permeable conformation. The ratio of the isomerization equilibrium constants with two and zero bound agonist molecules (E_2/E_0) is equal to the ratio of agonist affinities in the inactive vs. active conformations of the protein, at two identical binding sites [$(Kd/Jd)^2$]. We define R=Kd/Jd. Different agonists have different efficacies because they have different R values (E_0=6.5E-7). For ACh (=100 mV, 23 °C, mouse $\alpha_2\beta\delta\epsilon$), E_2=28.2 and R=6500. For choline, E_2=0.05 and R=270. For tetramethylammonium and carbachol, E_2=6.8 and R=3300. As part of a larger project to engineer the AChR transmitter binding site, we have measured E_2 and R values for a series of small molecular fragments. The goal is to generate an atomic map of the isomerization-induced energy changes at the ligand [kcal/mol=0.59ln(R)]. We first studied a series of five- and six- membered N-containing rings (fragments of nicotine). In the nicotine-bound AChBP structure, the pyrrolidine ring of the ligand is at the center of an aromatic 'box'. Systematic substitutions of the atoms of this ring are being made and the corresponding R-values are being determined. Preliminary work suggests that the following small molecules activate wild-type AChRs: 1,1-Dimethyl pyrrolidin-1-ium (E_2~0.26, R~630), 1,1-Dimethyl Thiazolidin-1-ium (E_2~0.68, R~1016) and 4,4-Dimethyl morpholinium (E_2~0.3, R~675).

686-Pos

Thinking Outside the Box: Residues that Shape the Agonist Binding Site of Nicotinic Acetylcholine Receptors

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Nicotinic acetylcholine receptors (nAChRs) are pentameric neurotransmitter-gated ion channels that mediate rapid synaptic transmission throughout the central and peripheral nervous systems. They are well-established targets for small molecule treatments for Alzheimer's disease, schizophrenia, Parkinson's disease, epilepsy, autism, and smoking cessation. To date, 17 human genes have been identified that code for nAChR subunits, termed α1-α10, β1-β4, γ, δ, and ϵ. nAChRs are subdivided into two main categories: the prototypical muscle-type receptor with a precise stoichiometry of $(\alpha1)_2\beta\delta\gamma$ (fetal form) and the ''neuronal'' nAChRs that are formed from various combinations of α2-α10 and β2-β4 subunits. Crystal structures of the acetylcholine binding protein have indicated that various ligands are positioned into a localized binding pocket formed from a cluster of conserved aromatic residues, termed the ''aromatic box''. Previous work from our lab has indicated that a common component of the neurotransmitter-binding occurs through the cationic center of the ligand and the face of an aromatic amino acid, termed the cation-π interaction, as well as hydrogen bonding interactions. Together, these binding events cause a change in protein structure permitting ion flow through the channel pore. In the immediate vicinity of the agonist binding site, all nAChR subtypes show identical amino acid compositions, yet significant pharmacological variations are seen. Previous work identified a point mutation, G152K, located near but not directly contributing to the agonist binding site in the α7 nAChR that is critical to agonist potency. Interestingly, this residue is a glycine in the low affinity receptors, such as the muscle-type and α7 nAChRs, but a lysine in the high affinity α4β2 nAChR. Here, we investigate the importance of this residue (G or K) in influencing acetylcholine and nicotine binding interactions in the muscle-type, α7 and α4β2 nAChRs.

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Efficient Isolation and Characterization of Nicotinic Acetylcholine Receptor from *Torpedo Californica* using Lipid Analog Detergents

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The effect of detergent solubilization on nicotinic acetylcholine receptor (nAChR) function has been extensively studied by several laboratories with the ultimate goal of characterizing the dynamic detergent-lipid-protein interactions of functional nAChR in both native and reconstituted membranes, as well as the detergent-solubilized state. These studies have provided substantial data on suitable detergents for solubilization, purification and functional reconstitution of the nAChR obtained from the electric organ of *Torpedo californica* electric rays. However, the molecular mechanisms by which particular detergents influence nAChR function remain poorly understood. In the present study, we characterized the effect of detergent solubilization and affinity column purification on *Torpedo* nAChR by using a series of lipid-like detergent with similar acyl composition to the most abundant fatty acid found in the native tissue of *Torpedo* (16:0, 18:0, 16:1 16:0)as well as cholesterol- analog detergents. . Fatty acid analogs included members of the Fos-choline (FC) family of detergents (FC-12, −14, −16 and lyso-FC), while cholesterol analogs were represented by cholate, taurocholate and CHAPS. Each detergent was used to s solubilize and purify the nAChR using established affinity column protocols, followed by analytical size exclusion chromatography (A-SEC) to probe the stability and aggregation state of the nAChR in solution, as well as planar lipid bilayers to probe ion channel function. The overall results showed that the

stability and function of the solubilized and purified receptor is preserved in lipid-analog detergents that have acyl chains similar to the most abundant lipid (16:0, 18:0, 16:1 16:0) in the endogenous *Torpedo* lipid environment, providing a suitable set of detergents for future studies. Supported by NIH grants, RISE, FIPI, 2RO1GM56371-12 and 2U54NS43011.

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Oligomeric Size and Configuration of the M_2 Muscarinic and β_2 Adrenergic Receptors in Live Cells as Determined by Pixel-Level FRET

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G protein-coupled receptors are known to form oligomers, but estimates of their size range from dimers to large arrays. We therefore have determined the oligomeric size of fluorophore-tagged M_2 and β_2 receptors in the plasma membrane of Chinese hamster ovary cells by examining the distribution of FRET efficiencies measured at the level of single pixels. Each receptor was fused at its *N*-terminus to enhanced green or yellow fluorescent protein and co-expressed as the complementary pair ($EGFP^2$-M_2 and EYFP-M_2, or $EGFP^2$-β_2 and EYFP-β_2). Pixel-level emission spectra were recorded from images captured in a single plane; the relative contribution of each fluorophore then was determined by spectral deconvolution and used to calculate the corresponding FRET efficiency. The distribution of efficiencies from each cell was analyzed as a sum of Gaussians. The number of Gaussians and the numeric relationship among the corresponding means (E) is determined by the number of combinatorial arrangements of FRET-productive pairs within a two-dimensional oligomer as predicted by the binomial theorem. A dimer will reveal a single efficiency or Gaussian, and a triangular trimer will reveal two; a square tetramer will reveal three efficiencies, and a rhombus will reveal five. Both the M_2 receptor and the β_2 receptor required five Gaussians to describe the distributions of efficiencies from several cells taken together. In each case, an efficiency (E) identified as the pair-wise efficiency (E_p) was related to the other four efficiencies in the manner predicted for a rhombus.

689-Pos

Anesthetic Binding Sites in the Neuronal N-Acetylcholine Receptor Transmembrane Domain

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Neuronal nicotinic acetylcholine receptors (nAChRs) are sensitive to general anesthetics at physiologically relevant concentrations, but the locations and mechanisms of relevant anesthetic interactions are unknown. The interactions of the anesthetics halothane, isoflurane, and ketamine with the transmembrane domains of the human nAChR α_4 and β_2 subunits were studied using fluorescence spectroscopy and high-resolution solution NMR. The isolated transmembrane domains, with the extracellular and intracellular domains removed by mutagenesis, were expressed in E. coli and purified into detergent micelles. Multi-angle light scattering experiments on a mixture of α_4 and β_2 subunits demonstrated their interaction in detergent micelles. Anesthetics quenched the intrinsic tryptophan fluorescence of these proteins in a manner consistent with specific binding. Direct interaction with anesthetic halothane, isoflurane, and ketamine was confirmed by NMR saturation transfer difference spectroscopy. The anesthetics induced chemical shift changes for specific resonances in the NMR spectra of the α_4 and β_2 transmembrane domains. Two specific interaction sites for the volatile anesthetics halothane and isoflurane have been identified at the extracellular and intracellular interfaces near the ends of the transmembrane domain in the α_4 subunit. Differing from halothane and isoflurane, the intravenous anesthetic ketamine affected a set of residues at a more interior location near the extracellular interface. Changes in NMR peak intensity and line width indicated modulations of protein motion by all three anesthetics. This study demonstrated that general anesthetics interact at specific sites within the isolated transmembrane domain of nAChR and affect protein dynamics on a time scale consistent with protein function. This work was supported by NIH grants: R01GM066358, R01GM056257, R37GM049202, and R01GM069766.

690-Pos

Effects of Isoflurane Binding in the Pore of a Ligand-Gated Ion Channel

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A high resolution homologue of the eukaryotic nicotinic acetylcholine receptor has been recently crystallized in an apparently open state from the bacteria *Gloeobacter violaceus* (Bocquet *et al*., Nature, 2008). Using the crystal structure of *Gloeobacter violaceus*' ligand-gated ion channel (GLIC) as a starting configuration, extensive molecular dynamics (MD) simulations were performed on the microsecond time scale for both a control and isoflurane-flooded system. This MD data reveal that isoflurane may diffuse from random positions within the water until it binds tightly in the pore to the M2 helices of GLIC. A similar result has also been observed in analogous simulations of the nicotinic receptor. The binding of isoflurane to the M2 helices in GLIC proceeds along a path into the channel via the large opening of the pore on the extracellular domain side. Analysis of the MD trajectory following isoflurane binding shows noticeable dehydration and decreased flexibility of the pore's solvent compared to the control system. Moreover, a close examination of the protein's backbone motions from both MD simulations suggests that presence of isoflurane dramatically increases the dynamics of the protein, in particular the dynamics of the individual residues in the M2-M3 loop. This combined data present a two-fold effect of isoflurane binding in the pore of GLIC: i) a statistically significant change in the dynamics occurs at nearby pore residues, and ii) ion conductance across the membrane decreases due to a change in the dynamical properties of the water in the pore.

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Molecular Dynamics Simulation Reveals a Possible Mechanism of Activation of the Prokaryotic Proton Activated Channel Glic

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Recent bioinformatics and high resolution structural determination revealed prokaryotic ancestors to the superfamily of eukaryotic Cys-loop receptors. One of these channels, called GLIC, is proton-activated and may provide advantages to investigating mechanisms underlying activation of Cys-loop receptors. Here, we used all-atom molecular dynamics simulations to uncover a possible mechanism of activation of the GLIC channel. We started with the x-ray structure of GLIC in the open state and subjected it to simulation under normal conditions. During the 10 ns simulation the channel appeared to close. Then we screened the apparent closed structure for the presence of salt-bridges, and compared the result to salt bridges in the original open x-ray structure. We found 11 salt bridges per subunit that were not present in the open structure. We then protonated the corresponding 11 acidic residues, conducted another 10 ns simulation and found that the channel remained open. We then extracted the final structural frames from the two MD simulations and employed single channel current simulations using the coarse-grained Biology Monte Carlo method. The open structure resulted in a significant ionic current, whereas the closed structure exhibited none. Thus, the 11 acidic residues emerge as likely candidates for mediating proton activation of the GLIC channel.

692-Pos

The Effect of a Hydration Pocket on the Function Of nAChR Studied by Computational Approach

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The first and solely available crystal structure of the extracellular domain of the nicotinic acetylcholine receptor (nAChR) was published in 2007 (Dellisanti et al. 2007). The intriguing finding of the paper was the existence of a hydration pocket inside the beta sandwich core of the nAChR. A well-ordered water molecule bonded by two hydrophilic residues, Thr52 and Ser126, was found. Based on the patch-clamp experiments of the wild-type and mutant receptors and the fact that the nonchannel homolog acetylcholine binding protein (AChBP) has bulky hydrophobic residues at these positions, authors assumed that hydration pocket might be a key element required for the receptor function as a ligand-gated ion channel. Although this assumption seems quite reasonable, computer simulation is required to provide the energetics of conformational changes.

The application of brute force MD simulations to the study of the above problem and other aspects of the action of nAChRs is problematic. This reflects the fact that the time scale for the gating transition is beyond the range of current computational capability. For example, nanoseconds-long MD simulations performed earlier (Law et al. 2005, Cheng et al. 2007) sample only the local conformational space of the initial channel's state. Thus we explored the origin of the hydrophilic/hydrophobic substitution effect by formulation this problem in terms of the relevant free energy changes and applying a proper thermodynamic cycle plus free energy perturbation calculations. Exploring the effect of the Thr52Val in both the open and closes states of nAChR appeared to provide an interesting microscopic insight on the operation of this system and highlighted the importance of using the proper computational strategies in studying biophysical problems.